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On the Functions of ABC Transporters in Plants

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Abstract

ATP Binding Cassette (ABC) proteins are ubiquitously found in prokaryotes and eukaryotes and generally serve as membrane-intrinsic primary active pumps. In higher plants, ABC proteins constitute a large family, phylogenetically structured into eight clusters, namely the ABCA to ABCI subfamilies [1]. ABC transporters shuttle substrates as diverse as lipids, phytohormones, carboxylates, heavy metals, chlorophyll catabolites and xenobiotic-conjugates across a variety of biological membranes. To date, the largest proportions of characterized members have been localized to the plasma membrane and the tonoplast with dominant implications in cellular secretion and vacuolar sequestration, but they are also found in mitochondrial, plastidal and peroxisomal membranes. Originally identified as tonoplast-intrinsic proteins that shuttle xenobiotic-conjugates from

the cytosol into the vacuole, thus being an integral part of the detoxification machinery, ABC transporters are now recognized to participate in a multitude of physiological processes that allow the plant to adapt to changing environments and cope with biotic and abiotic stresses.

1. Introduction

The plant ABC protein family consists of full-size, half-size and several soluble members that all share a cytosolic nucleotide-binding domain. Functional transport units utilize ATP-hydrolysis to energize the transport of solutes across membranes, independent of concentration gradients and membrane potentials. Full-size members are organized in a modular fashion, consisting of two pore-forming transmembrane domains alternating with two cytosolic nucleotide-binding domains. Half-size members, which contain one transmembrane domain and one nucleotide-binding domain, are thought to form dimers that act as functional units. In most subfamilies, transmembrane domains precede nucleotide-binding domains, a topology which is referred to as forward orientation, while the opposite is considered a reverse orientation [2]. An additional N-terminal transmembrane domain (TMD0) is characteristic to members of the ABCC subfamily. In humans and yeast, the TMD0 of some ABCC proteins was demonstrated to be essential for correct protein targeting, without necessarily having implications in substrate specificity and transport activity [3,4]. The plant soluble ABC proteins cluster in the ABCE, ABCF and ABCI subfamilies. However, due to a primary focus on membrane-intrinsic half- and full-size proteins, they will

not be addressed further in this review. Readers interested in this topic will find information in two comprehensive reviews on plant ABC proteins [2,5].

Genome wide inventories of ABC transporters in Arabidopsis, rice and poplar led to the identification of more than 100 loci encoding for either half-size or full-size transporters in each, making them nearly twice as numerous in plants as reported for sequenced animal species. Half-size (WBC) and full-size (PDR) members of the reverse oriented ABCG subfamily are abundantly found in plants. No full-size ABCG transporters have been identified in animals. It is argued that the expansion and diversification of the ABC protein family in plants may be tightly linked to their autotrophic and sessile lifestyle, requiring a highly plastic development, interaction with the environment and maintenance of basic metabolism under changing conditions.

As a secondary effect of nutrient acquisition from the soil, plants often take up toxic heavy metals, which need to be secreted in order not to interfere with metabolism. One possibility is reflux into the soil or secretion into the foliar apoplast. Full-size ABCG members have been identified as candidates for cadmium and possibly lead export [6,7]. Furthermore, ABC transporters play an important role in the general detoxification mechanism. Extrinsic or intrinsic potentially toxic compounds are first incorporated into conjugates, which are then recognized by tonoplastic ABC transporters and sequestered into the vacuole [8].

Phytohormones are major modulators of plant development and plant responses to biotic and abiotic environmental stimuli. Sites of hormone production differ from sites of hormone action and thus, export from producing cells, directed

distribution within the plant body and specific uptake into receptive cells are crucial for adequate plant function. ABC transporters participate in the translocation of the hormone auxin, a dominant developmental regulator [9], and the hormone abscisic acid (ABA), implicated in abiotic stress responses [10,11].

Plants possess an often species specific array of natural compounds that are produced and deposited in a strictly regulated manner to directly interact with the biotic environment such as deterring herbivores, warding off pathogens and attracting symbionts or pollinators. Their biosynthetic pathways often involve several organelles within a cell and different cell types within an organism. Precursors as well as final products need to be transported across membranes and in several cases ABC transporters are proposed as candidates. Best studied in this respect is the abaxial epidermal deposition of antimicrobial compounds [12,13].

2. Cellular Detoxification

First indications for ABC-mediated transport processes in plants were observed when Martinoia *et al*. [14] began investigating how xenobiotics are detoxified in plants. They found that potentially toxic compounds, bound to the tripeptide glutathione through their thiol group (glutathione conjugates), are transported into the vacuole independently from the proton motive force generated by two vacuolar proton pumps. Initially, detoxification processes in plants and animals are very similar, involving cytochrome P450 proteins and several transferases, such as glutathione S-transferases, glucuronosyltransferases or

93 glycosyltransferases [15]. Unlike animals which excrete modified, potentially toxic
94 compounds, plants internalize toxins in large central vacuoles. Two vacuolar
95 glutathione conjugate transporters, AtABCC1/AtMRP1 and AtABCC2/AtMRP2,
96 were identified [16]. Both proteins transport a broad range of glutathione
97 conjugates, glucuronates as well as a chlorophyll catabolite. Later studies have
98 shown that AtABCC1 is an efficient transporter of folates, which are known to be
99 stored in the vacuole and are required for methylation processes [17]. The latter
100 findings suggest that ABCCs are not solely involved in cellular detoxification.
101 Proteomic data obtained for Arabidopsis vacuoles suggest that most ABCCs of
102 Arabidopsis reside in the tonoplast [18], and other ABCCs, additionally to
103 AtABCC1/2, may contribute to the overall glutathione conjugate transport activity.
104 Heavy metals are also stored within the central vacuole for detoxification
105 purposes and data suggest that phytochelatins and phytochelatin heavy metal
106 complexes are transported by ABCC-type transporters [19]. In contrast to *S.*
107 *cerevisae*, where an ABCC-type transporter, YCF1 (yeast cadmium factor 1),
108 catalyzes the transport of glutathion-cadmium complexes [20], and to *S. pombe,*
109 where Hmt1, a half-size ABC transporter, sequesters phytochelatin [21], the
110 nature of the plant-specific transporter for phytochelatin or heavy metal
111 glutathione complexes is unknown. Some ABCCs can partially rescue the heavy
112 metal sensitivity of the ycf1 yeast mutant [22], but the substrate transported has
113 not been identified.
114 A member of the AtABCG family, the half-size AtABCG19, confers kanamycin
115 resistance when overexpressed in plants [23]. Since AtABCG19 is highly specific

for kanamycin transport and does not confer resistance to other aminoglycoside antibiotics of clinical importance, the authors propose AtABCG19 as a novel resistance marker for the creation of transgenic plants. Not only would AtABCG19 constitute an endogenous plant-derived marker, it would also limit resistance properties to the antibiotic kanamycin, were it to be transferred horizontally to potentially pathogenic microorganisms.

An alternative way for plants to cope with toxic compounds, soilborne heavy metals in particular, is secretion from the cell, aboveground into the apoplast or cuticle and belowground into the rhizosphere. In Arabidopsis, the plasma membrane-intrinsic full-size AtABCG36/AtPDR8, previously described as pathogen defense related (see also chapter 4.), is involved in the detoxification process [7]. Based on the observation that *AtABCG36* expression was promoted by exogenously applied cadmium and lead, the authors tested the response of wild type plants, *Atabcg36* knock-out mutants, *AtABCG36* silenced mutants and *AtABCG36* over-expression mutants to elevated heavy metal concentrations. A clear positive correlation between transcript abundance and resistance to lead and cadmium was established. Furthermore, with respect to the wild type, over-expression lines accumulated less cadmium, and silenced lines more cadmium in root and shoot tissues. Direct involvement of AtABCG36 in the export of cadmium ions or cadmium complexes was demonstrated utilizing isolated mesophyll protoplasts in a ^{109}Cadmium flux assay. Whereas an over-expressing line proved more efficient in extruding ^{109}Cadmium than the wildtype, a silenced

138 line was impaired in its export capacities. The form in which the toxic heavy metal
139 cadmium is transported, whether as free ion or in a chelated form, is unknown.
140
141 **3. Growth and Development**
142
143 **3.1. Phytate Transport**
144 Inositol hexa*kis*phosphate ($InsP_6$, phytate) constitutes a major phosphorus store
145 in plants. It accumulates predominantly in seeds as a complex with magnesium,
146 potassium, calcium, iron and/or zinc, representing up to 5% of the seed dry-
147 weight [24,25]. During germination phytate is degraded by phytases, thus
148 providing phosphate, myo-inositol and minerals to developing seedlings. Recent
149 publications revealed that $InsP_6$ fulfils additional physiological roles. Plants
150 lacking $InsP_6$ are more susceptible to pathogen attack [26], while in guard cells
151 $InsP_6$ mobilizes calcium from endomembrane stores and inhibits the inward
152 rectifying K^+ conductance, thereby influencing stomatal movements [27,28]. $InsP_6$
153 can have a negative impact on the environment, since monogastric animals that
154 lack phytases in their digestive tract fail to process the phytates present in seeds.
155 As a consequence, high amounts of undigested phytates are released with the
156 animal waste into nature, thus accentuating the phosphorus pollution from
157 agriculture [29].
158 During the last few years, low phytate mutants were described in major crop
159 species, *i.e.* maize, rice and sorghum [30-33]. While in most cases a 45-90%
160 reduction in seed phytate content did not result in detectable phenotypes, some

161 of the rice mutant lines proved nonviable. In maize, detailed genetic analysis led
162 to the identification of the ABCC-type transporter *ZmMRP4* as the responsible
163 gene behind $InsP_6$ accumulation in kernels [30]. Phylogenetic studies identified
164 the tonoplastic AtABCC5/AtMRP5 as the closest Arabidopsis homologue to
165 ZmMRP4. *Atabcc5* loss-of-function mutants display a low $InsP_6$ phenotype in
166 seed tissue, which is associated with alterations of mineral cation and phosphate
167 status [34]. Heterologous expression in yeast demonstrated that AtABCC5
168 encodes a specific high affinity $InsP_6$ transporter. Moreover, complementation of
169 the *Atabcc5* knock-out mutants with an At*ABCC5* construct driven by a guard
170 cell-specific promoter restored the sensitivity of the mutant to ABA–mediated
171 inhibition of stomatal opening, supporting the hypothesis that $InsP_6$ acts as a
172 signaling molecule in guard cells. Hence, it is proposed that by transporting $InsP_6$
173 into the vacuole, AtABCC5 is essential for the regulation of correct stomatal
174 movement as well as for phytate storage in seeds. One could anticipate that the
175 elucidation of the $InsP_6$ transport mechanism will contribute to the understanding
176 of the regulation of various cellular signalling processes. From an applied
177 perspective it could furthermore address nutritional and environmental questions
178 regarding the possibility of enhancing the quality of seeds by altering the $InsP_6$
179 content.
180
181 **3.2. Import of fatty acids into peroxisomes**
182 Beta-oxidation of fatty acids is a very important catabolic process, required to
183 generate Acetyl-CoA for entry into the citric acid cycle. In plants it occurs

predominantly within the peroxisomes and thus it requires that fatty acid-CoAs are imported from the cytosol. Arabidopsis loss-of-function mutants of AtABCD1, a full-length peroxisomal ABC transporter, are strongly impaired in germination and display a pleiotropic growth phenotype. These mutants furthermore exhibit an impaired fatty acid metabolism, suggesting that AtABCD1 is the fatty acid-CoA importer in plant peroxisomes [35-37].

3.3. Hormone Transport

Plant development and adaptation to the environment are closely linked to the action of phytohormones. Trytophan derived indole-3-acetic-acid (auxin) is a central phytohormone, implicated in many developmental processes such as shoot elongation, floral bud development, lateral root growth, phototropism and gravitropism [38]. It is mainly produced in the shoot apical meristem, from where it is distributed throughout the plant via cell to cell transport within the xylem parenchyma [38]. Cellular auxin import can either occur simply via diffusion of its protonated form or is catalyzed by members of the AUX1/LAX family of auxin-influx carriers [39]. Polar export was thought to be governed solely by the PIN family of auxin transporters [39]. However, a detailed examination of two plasma membrane-intrinsic ABCB transporters, AtABCB1 and AtABCB19, suggested that ABC transporters also contribute to inter-cellular auxin transport [40]. *Atabcb1* and *Atabcb19* knock-out plants exhibited slightly retarded growth patterns, while the corresponding double knock-out was strongly impaired in growth, phenotypically reminiscent of auxin deficiency symptoms. Furthermore, auxin

207 transport in the double knock-out mutant was reduced by more than 70%.
208 Heterologous expression of AtABCB1 and AtABCB19 in several systems
209 presented direct evidence that both proteins are indeed auxin exporters. Recent
210 studies suggest that the two auxin efflux systems are at least partially
211 interconnected. PIN proteins and ABC transporters interact to modulate the
212 overall auxin transport activity in a complex fashion [41,42]. Furthermore, it was
213 shown that the immunophilin-like protein TWD1 interacts with ABCBs,
214 significantly stimulating auxin transport [43,44]. In contrast to most members of
215 the PIN family, AtABCB1 and AtABCB19 are expressed only in specific cell types.
216 This, in addition to functional overlaps with PIN proteins, may explain why auxin
217 specific ABCB mutant phenotypes are restricted to impairments in cell elongation
218 of shoot and root.
219 Two recent publications reported that ABC transporters are involved in the
220 transport of the carotenoid-derived stress hormone ABA. Besides being a
221 germination inhibitor present in dormant seeds, ABA is mainly produced in the
222 vasculature of shoots and roots as a response to hydric stress [45,46]. From
223 there it is translocated to foliar tissues, where it induces stomata closure to
224 minimize water loss. Consequently, ABA has to be exported from xylem
225 parenchyma cells, transferred to leaves and imported into guard cells and other
226 cell types to trigger the signaling pathways required for coping with water stress.
227 A half-size transporter in the ABCG family, AtABCG25, is expressed in the
228 vascular parenchyma of root and shoot and was shown to act as an ABA
229 exporter with a high affinity for ABA (apparent Km of 0.2 µM) in heterologous

transport systems [10]. While germination of the corresponding mutant was more sensitive to the application of exogenous ABA, no stomata-specific phenotype could be observed. However, overexpressing AtABCG25 resulted in plants that transpired less water, indicating enhanced ABA export capacities from producing cells. A full-size transporter, AtABCG40, on the other hand, previously proposed to be implicated in heavy metal resistance [6], was found to contribute to the import of ABA across the stomatal plasma membrane [11]. Stomata of *Atabcg40* mutant plants close less efficiently when the roots are exposed to ABA and the upregulation of ABA-responsive genes upon ABA treatment is delayed. As a consequence, mutant *Atabcg40* plants are more sensitive to drought stress. In heterologous systems, AtABCG40 imports ABA with an apparent Km of 1 µM, which is within the expected physiological range. Still, the exact mechanism by which AtABCG40 achieves the import of its substrate has yet to be investigated. Import mechanisms are well known from bacterial ABC transporters, which catalyze the uptake of nutrients [47]. In contrast, no ABC importers have been described in the animal field. In plants several full-size ABC transporters have been proposed to act as importers (see also chapter 3.2.). Similarly to AtABCG40, AtABCB14 resides in the stomatal plasma membrane, where it is suggested to govern over the import of apoplastic malate, thus influencing stomatal movements in response to changing carbon dioxide concentrations [48]. An interesting case is AtABCB4, where evidence was presented that, depending on the auxin concentration in the cytosol or medium, it can act as importer or

252 exporter [49]. Further studies concerning structural aspects are pending to
253 unravel the details of ABC-mediated import in plants.
254
255 **3.4. Cuticle Formation**
256 The plant cuticle is primarily composed of cuticular waxes (aliphatic very long
257 chain fatty acid derivatives), embedded in a cutin matrix (mainly glycerol, C16
258 and C18 fatty acids) [50]. It covers the epidermis of aerial organs of land plants,
259 forming a protective layer against desiccation and pathogen entry. Proper cuticle
260 deposition is furthermore necessary for organ development, preventing organ
261 fusion and influencing organ morphology. Two half-size ABCG/WBC-proteins,
262 AtABCG11/WBC11 and AtABCG12/WBC12 of Arabidopsis, were demonstrated
263 to play a dominant role in cuticle formation [51,52].
264 AtABCG12, discovered in a forward screen for wax lacking mutants, is expressed
265 in the epidermal tissues of most plant organs and localizes to the plasma
266 membrane, where it is proposed to play a direct role in the export of a multitude
267 of wax precursors [51]. Stem epidermal cells of *Atabcg12* knockout plants feature
268 laminar cytoplasmic inclusions of lipidic nature and the stem cuticle is depleted in
269 a variety of wax components such as long chain alkanes, ketones and alcohols,
270 giving the stem a glossy bright green appearance. In contrast, the total amount of
271 epidermal wax, intracellular and cuticular, does not differ between WT and
272 mutant, suggesting that the cytosolic inclusions comprise an accumulation of wax
273 components that are lacking in the cuticle due to transport defects.

AtABCG11 transcript is predominantly found in the epidermis of aerial organs. The protein localizes to the plasma membrane, with a marked polar distribution on the distal epidermal side in developing embryos [53]. In congruence with AtABCG12 function, it contributes to the secretion of cuticular waxes and loss-of-function results in an aberrant cuticle structure [52]. In contrast to *Atabcg12*, *Atabcg11* also displays a reduced cutin load, suggesting cutin precursor fatty acids as additional substrates. The cuticle is one of the main barriers against non-stomatal water loss. Thus, it was intriguing to find that the water stress related hormone ABA induces upregulation of *AtABCG11* [54], which is speculated to enhance fortification of aerial organs against desiccation. Consistent with this finding, *Atabcg11* mutants are prone to wilting, but more conspicuously, they display a pleiotropic growth phenotype, with a marked reduction in growth, fusion of rosette leaves, altered reproductive organs and sterility.

Double mutants of *AtABCG12* and *AtABCG11* do not exhibit an additive phenotype [55]. Consequently it was suggested that they form heterodimers, with a strong affinity for cuticular waxes. The additional cutin phenotypes of *Atabc11* mutants suggest that AtABCG11 can also form homodimers or heterodimers with half-size ABCG members other than AtABCG12 to create a complex with a high affinity for cutin precursor fatty acids.

4. Pathogen Defense

296 Anti-microbial plant secondary metabolites such as terpenoid derivates and
297 cyanogenic glycosides form an important first line of defense against host and
298 non-host pathogens [56]. They inhibit the proliferation of fungal and bacterial
299 microbes on aerial plant surfaces, within the rhizosphere and in the apoplast
300 around local infection sites. There is increasing evidence that the aboveground
301 and belowground secretion of such compounds is in part mediated by full-size
302 ABC transporters of the ABCG/PDR subfamily. Transcript profiling of ABCG
303 members in rice [57] revealed that nearly half of them positively respond to
304 jasmonic acid (JA) and/or salicylic acid (SA), two phytohormones implicated in
305 biotic stress responses. This suggests roles in pathogen defense, which is
306 supported by the finding that three full-length *ABCG*s are induced in rice leaves
307 that are infected with the biotrophic fungus *Magnaporthe grisea*. Belowground,
308 loss-of-function of AtABCG30/AtPDR2 results in a drastic change of soil
309 microflora in the rhizosphere [58], which the authors attribute to a change in root-
310 exudate composition. Additionally, the overall profile of root exudates is altered in
311 several ABC transporter mutants [59].
312 Particularly within the rhizosphere, plant-derived secondary compounds serve as
313 powerful attractants for beneficial microbes such as mycorrhizal fungi and
314 rhizobacteria. Specific inhibitor studies suggest that ABCG subfamily members
315 are responsible for the secretion of genistein and daidzein, both iso-flavonoids
316 that act as plant-derived signaling molecules in the legume-rhizobia symbiosis,
317 into the rhizosphere [60,61].

Functional characterization of NpPDR1, a full-size ABCG protein of *Nicotiana plumbaginifolia*, was the first report of a plant ABC transporter being implicated in pathogen defense and the first description of active terpenoid transport in plants [62]. NpPDR1 resides in the plasma membrane and is induced by the anti-fungal diterpenes sclareol and sclareolide. In isolated microsomes NpPDR1 contributes to the transport of radiolabeled compounds closely related to sclareol, supporting the hypothesis that sclareol is a natural substrate. *NpPDR1* transcript is most abundant in the leaf epidermis, including leaf trichomes, but is also present in root tissues and petals [13]. Apart from its proposed substrate sclareol, both JA and SA promote *NpPDR1* expression, indicating association with defense-related signaling pathways. Concomitantly, a strong response to *Botritis cinerea* and *Pseudomonas syringae*, both necrotrophic non-host pathogens, is observed. Downregulation of NpPDR1 lead to spontaneous and commonly lethal infections with *B. cinerea* and rendered the plant highly susceptible to exogenously applied sclareol. All these findings indicate a participation of NpPDR1 in basal plant defense. It is hypothesized that antimicrobial compounds such as sclareol are deposited via NpPDR1 on the leaf surface and possibly exuded into the rhizosphere, contributing to a constitutive chemical defense barrier. Upon perception of various pathogens and transduction via JA and SA dependent pathways this mode of defense is intensified locally around areas of infection, so that NpPDR1-mediated modes of action are both constitutive and induced.

AtABCG36/AtPDR8/PEN3 was first recognized as a crucial factor in pre-invasive nonhost resistance in an extensive forward genetic screen of Arabidopsis

341 mutants for an increased susceptibility to the barley powdery mildew pathogen
342 [12]. The *AtABCG36* loss-of-function mutants were compromised in their capacity
343 to prevent entry of two nonhost biotrophs and one nonhost necrotroph. However,
344 they proved hyperresistant against the compatible Arabidopsis powdery mildew
345 pathogen, which the authors attribute to a hyperactivation of SA-dependent
346 pathways observed in the mutant. GFP-fusion construct under the control of the
347 native promoter complemented the phenotype and the corresponding fusion
348 protein was targeted to the plasma membrane, with increased fluorescence
349 intensities at infection sites. The authors hypothesize that AtABCG36 transports
350 defense related compounds across the plasma membrane in a concentrated
351 manner at local infection sites. Lack of AtABCG36 function would lead to an
352 accumulation of these compounds within the cell, which may activate SA
353 dependent pathways that prime defense against compatible pathogens.
354 Recently a full-size *ABCG* was identified as the responsible gene behind a robust
355 and durable pathogen resistance against leaf rust, stripe rust and powdery
356 mildew in wheat carrying functional *LR34* (Leaf Rust 34) alleles [63]. Unlike many
357 other ABCGs, its expression seems to be modulated by developmental cues
358 rather than stress factors. LR34 is predominantly found in adult foliar tissues,
359 particularly the flag leaf. Transcript abundance is highest in the leaf tip and wheat
360 varieties with functional *LR34* alleles can be phenotypically selected via a leaf tip
361 necrosis developing in adult flag leaves. Despite its resistance conferring
362 properties, LR34 is not responsive to pathogen inoculation, suggesting rather
363 constitutive than induced functions. In contrast to NpPDR1 and AtABCG36

364 function, which seem restricted to nonhost resistance, LR34 is implicated in the
365 defense against several compatible pathogens of fungal origin. LR34 is the only
366 ABCG protein characterized to date, that impedes the invasion and spread of
367 compatible pathogens. Research with focus on the nature of its substrates is
368 ongoing.
369 Considering the participation of ABCG transporters in secondary metabolite-
370 based pathogen response, it is reasonable to assume that they also play a role in
371 herbivory defense, *e.g.* via the deposition of insect-deterring compounds on leaf
372 surfaces. Certain compounds of terpenoid origin are known to act as herbivore
373 deterrents and JA, a potent inducer of many full-size ABCGs, is also a major
374 mediator of herbivory responses.
375
376 **5. Conclusion**
377 The functional study of plant ABC transporters is a rapidly expanding field. It is
378 becoming increasingly apparent that ABC dependent transport processes not
379 only serve to protect the plant from endogenous and exogenous toxic
380 compounds, but that they are indispensable for proper development, adequate
381 interaction with the environment and basic metabolic processes.
382 There seems to be no clear functional separation among the different subfamilies,
383 but from our current knowledge it is possible to define subfamily specific
384 preferences. Members of the ABCB subfamily have been mainly implicated in
385 organic acid transport across the plasma membrane and it is probable that other
386 members serve similar functions, *e.g.* in the excretion of carboxylates for either

cytosolic pH homeostasis or rhizosphere acidification. Secondary compounds of terpenoid origin and highly lipophilic compounds are common substrates for members of the ABCG clade. The question remains whether ABCG proteins might also display specificity for other secondary compound classes such as alkaloids and flavonoids. Biotechnological approaches to produce natural compounds of medicinal value in heterologous or modified tissue culture systems are currently pursued with emphasis to supply the pharmaceutical market. Understanding and designing highly productive systems must necessarily include efficient transport mechanisms to relocate precursors within the manufacturing units and to excrete final products into the medium. The ability of several subfamily members to efficiently sequester xenobiotic compounds and heavy metals is also of importance to the biotechnological market. Attempts to detoxify contaminated soils via phytoremediation, utilizing genetically modified plants, are underway. Altered expression of certain ABC transporters appears to be part of a feasible approach, for example, to prevent reflux of extracted contaminants into the soil and enhance vacuolar accumulation in foliar tissues.

The novel findings that ABCG members are participating in ABA transport are intriguing and tempt us to speculate that the translocation of hormones other than ABA and auxin might also rely on ABC-dependent mechanisms. Possible candidates falling into the predicted substrate range of ABCGs are the recently discovered branching hormone strigolactone [64,65], an apocarotenoid structurally related to ABA, and the brassinosteroids, highly lipophilic terpenoid derivates.

ABC proteins play an important role in plant growth and development. The function of most of plant ABC proteins still awaits elucidation. Functional redundancies render classical mutagenesis-based forward- or reverse-genetic approaches futile. Producing multiple loss-of-function mutants of closely related ABC transporters, and/or finding their interacting partners might reveal additional functions that will direct the studies on plant ABC transporters.

6. Summary

- Higher plants contain more than one hundred ABC proteins that are organized in eight clusters, namely ABCA to ABCI subfamilies, and predominantly localize to the plasma membrane and tonoplast.

- Most ABCCs/MRPs localize to the tonoplast where several of them are thought to catalyze the vacuolar sequestration of xenobiotic-conjugates.

- AtABCG36 plays a role in heavy metal tolerance, as well as in pathogen defense, but the nature of the respective substrates has yet to be determined.

- AtABCC5 is a tonoplastic phytate transporter with implications in phytate storage in seeds and regulation of stomatal movement.

- Several ABCBs/PGPs are participating in a directional cell to cell transport of auxin via polar export and interaction with other auxin transporters.

- Two ABCGs contribute to the inter-cellular transport of the apocarotenoid stress hormone ABA.

- Two half-size ABCGs/WBCs contribute to cuticle formation via the epidermal excretion of cuticular wax components.

- Several full-size ABCGs/PDRs have been implicated in pathogen defense supposedly via excretion of anti-microbial secondary metabolites.

- AtABCB14 localizes to the plasma membrane of guard cells and catalyzes the import of malate, thus influencing stomatal movement.

7. References

1. Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu Ü, Lee Y, Martinoia E, et al. (2008) Plant ABC proteins - a unified nomenclature and updated inventory. Trends in Plant Science **13**,151-159.
2. Rea PA (2007) Plant ATP-binding cassette transporters. Annu Rev Plant Biol **58**,347-375.
3. Mason DL, Michaelis S (2002) Requirement of the N-Terminal Extension for Vacuolar Trafficking and Transport Activity of Yeast Ycf1p, an ATP-binding Cassette Transporter
10.1091/mbc.E02-07-0405. Mol. Biol. Cell **13**,4443-4455.
4. Westlake CJ, Cole SPC, Deeley RG (2005) Role of the NH2-terminal membrane spanning domain of multidrug resistance protein 1/ABCC1 in protein processing and trafficking. Mol Biol Cell **16**,2483-2492.

5. Yazaki K, Shitan N, Sugiyama A, Takanashi K: Chapter 6 Cell and Molecular Biology of ATP-Binding Cassette Proteins in Plants. International Review of Cell and Molecular Biology. edn Volume 276. Edited by Jeon KW: Academic Press; 2009:263-299.
6. Lee M, Lee K, Lee J, Noh EW, Lee Y (2005) AtPDR12 contributes to lead resistance in Arabidopsis. Plant Physiol **138**,827-836.
7. Kim D-Y, Bovet L, Maeshima M, Martinoia E, Lee Y (2007) The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J **50**,207-218.
8. Klein M, Burla B, Martinoia E (2006) The multidrug resistance-associated protein (MRP/ABCC) subfamily of ATP-binding cassette transporters in plants. FEBS Lett **580**,1112-1122.
9. Geisler M, Murphy AS (2006) The ABC of auxin transport: the role of p-glycoproteins in plant development. FEBS Lett **580**,1094-1102.
10. Kuromori T, Miyaji T, Yabuuchi H, Shimizu H, Sugimoto E, Kamiya A, Moriyama Y, Shinozaki K (2010) ABC transporter AtABCG25 is involved in abscisic acid transport and responses. Proc Natl Acad Sci U S A **107**,2361-2366.
11. Kang J, Hwang J-U, Lee M, Kim Y-Y, Assmann SM, Martinoia E, Lee Y (2010) PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc Natl Acad Sci U S A **107**,2355-2360.
12. Stein M, Dittgen J, Sanchez-Rodriguez C, Hou B-H, Molina A, Schulze-Lefert P, Lipka V, Somerville S (2006) Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell **18**,731-746.
13. Stukkens Y, Bultreys A, Grec S, Trombik T, Vanham D, Boutry M (2005) NpPDR1, a pleiotropic drug resistance-type ATP-binding cassette transporter from Nicotiana plumbaginifolia, plays a major role in plant pathogen defense. Plant Physiol **139**,341-352.
14. Martinoia E, Grill E, Tommasini R, Kreuz K, Amrhein N (1993) ATP-dependent glutathione S-conjugate 'export' pump in the vacuolar membrane of plants. **364**,247-249.
15. Kreuz K, Tommasini R, Martinoia E (1996) Old Enzymes for a New Job (Herbicide Detoxification in Plants). Plant Physiol **111**,349-353.
16. Lu YP, Li ZS, Drozdowicz YM, Hortensteiner S, Martinoia E, Rea PA (1998) AtMRP2, an Arabidopsis ATP binding cassette transporter able to transport glutathione S-conjugates and chlorophyll catabolites: functional comparisons with Atmrp1. Plant Cell **10**,267-282.
17. Raichaudhuri A, Peng M, Naponelli V, Chen S, Sanchez-Fernandez R, Gu H, Gregory JF, Hanson AD, Rea PA (2009) Plant Vacuolar ATP-binding Cassette Transporters That Translocate Folates and Antifolates in Vitro and Contribute to Antifolate Tolerance in Vivo. J Biol Chem **284**,8449-8460.
18. Jaquinod M, Villiers F, Kieffer-Jaquinod S, Hugouvieux V, Bruley C, Garin J, Bourguignon J (2007) A Proteomics Dissection of Arabidopsis thaliana Vacuoles Isolated from Cell Culture. Mol Cell Proteomics **6**,394-412.

19. Salt DE, Rauser WE (1995) MgATP-Dependent Transport of Phytochelatins Across the Tonoplast of Oat Roots. Plant Physiol **107**,1293-1301.
20. Szczypka MS, Wemmie JA, Moye-Rowley WS, Thiele DJ (1994) A yeast metal resistance protein similar to human cystic fibrosis transmembrane conductance regulator (CFTR) and multidrug resistance-associated protein. J Biol Chem **269**,22853-22857.
21. Ortiz DF, Kreppel L, Speiser DM, Scheel G, McDonald G, Ow DW (1992) Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter. EMBO J **11**,3491-3499.
22. Tommasini R, Vogt E, Fromenteau M, Hortensteiner S, Matile P, Amrhein N, Martinoia E (1998) An ABC-transporter of Arabidopsis thaliana has both glutathione-conjugate and chlorophyll catabolite transport activity. Plant J **13**,773-780.
23. Mentewab A, Stewart CN (2005) Overexpression of an Arabidopsis thaliana ABC transporter confers kanamycin resistance to transgenic plants. Nat Biotechnol **23**,1177-1180.
24. Raboy V (2007) The ABCs of low-phytate crops. Nat Biotechnol **25**,874-875.
25. Raboy V (2003) myo-Inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry **64**,1033-1043.
26. Murphy AM, Otto B, Brearley CA, Carr JP, Hanke DE (2008) A role for inositol hexakisphosphate in the maintenance of basal resistance to plant pathogens. Plant J **56**,638-652.
27. Lemtiri-Chlieh F, MacRobbie EA, Brearley CA (2000) Inositol hexakisphosphate is a physiological signal regulating the K+-inward rectifying conductance in guard cells. Proc Natl Acad Sci U S A **97**,8687-8692.
28. Lemtiri-Chlieh F, MacRobbie EAC, Webb AAR, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA (2003) Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc Natl Acad Sci U S A **100**,10091-10095.
29. Coffey GCaR: Phosphorus—a key essential nutrient, yet a possible major pollutant—its central role in animal nutrition. In *Lyons, T. P. eds. Biotechnology in the Feed Industry*. Edited by; 1991:133-145.
30. Shi J, Wang H, Schellin K, Li B, Faller M, Stoop JM, Meeley RB, Ertl DS, Ranch JP, Glassman K (2007) Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. Nat Biotechnol **25**,930-937.
31. Shi J, Wang H, Wu Y, Hazebroek J, Meeley RB, Ertl DS (2003) The maize low-phytic acid mutant lpa2 is caused by mutation in an inositol phosphate kinase gene. Plant Physiol **131**,507-515.
32. Rasmussen SK, Hatzack F (1998) Identification of two Low-Phytate Barley (*Hordeum Vulgare*) Grain Mutants by TLC and Genetic Analysis. Hereditas **129**,107-112.
33. Xu X-H, Zhao H-J, Liu Q-L, Frank T, Engel K-H, An G, Shu Q-Y (2009) Mutations of the multi-drug resistance-associated protein ABC transporter

552 gene 5 result in reduction of phytic acid in rice seeds. Theor Appl Genet
553 **119**,75-83.
554 34. Nagy R, Grob H, Weder B, Green P, Klein M, Frelet-Barrand A, Schjoerring
555 JK, Brearley C, Martinoia E (2009) The Arabidopsis ATP-binding cassette
556 protein AtMRP5/AtABCC5 is a high affinity inositol hexakisphosphate
557 transporter involved in guard cell signaling and phytate storage. J Biol
558 Chem **284**,33614-33622.
559 35. Footitt S, Slocombe SP, Larner V, Kurup S, Wu Y, Larson T, Graham I, Baker
560 A, Holdsworth M (2002) Control of germination and lipid mobilization by
561 COMATOSE, the Arabidopsis homologue of human ALDP. EMBO J
562 **21**,2912-2922.
563 36. Hayashi M, Nito K, Takei-Hoshi R, Yagi M, Kondo M, Suenaga A, Yamaya T,
564 Nishimura M (2002) Ped3p is a peroxisomal ATP-binding cassette
565 transporter that might supply substrates for fatty acid beta-oxidation. Plant
566 Cell Physiol **43**,1-11.
567 37. Zolman BK, Silva ID, Bartel B (2001) The Arabidopsis pxa1 mutant is
568 defective in an ATP-binding cassette transporter-like protein required for
569 peroxisomal fatty acid beta-oxidation. Plant Physiol **127**,1266-1278.
570 38. Vanneste S, Friml J (2009) Auxin: a trigger for change in plant development.
571 Cell **136**,1005-1016.
572 39. Kramer EM (2004) PIN and AUX/LAX proteins: their role in auxin
573 accumulation. Trends in Plant Science **9**,578-582.
574 40. Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V,
575 Bandyopadhyay A, Titapiwatanakun B, Peer WA, Bailly A, Richards EL, et
576 al. (2005) Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP
577 transporter AtPGP1. Plant J **44**,179-194.
578 41. Bandyopadhyay A, Blakeslee JJ, Lee OR, Mravec J, Sauer M,
579 Titapiwatanakun B, Makam SN, Bouchard R, Geisler M, Martinoia E, et al.
580 (2007) Interactions of PIN and PGP auxin transport mechanisms. Biochem
581 Soc Trans **35**,137-141.
582 42. Blakeslee JJ, Bandyopadhyay A, Lee OR, Mravec J, Titapiwatanakun B,
583 Sauer M, Makam SN, Cheng Y, Bouchard R, Adamec J, et al. (2007)
584 Interactions among PIN-FORMED and P-glycoprotein auxin transporters
585 in Arabidopsis. Plant Cell **19**,131-147.
586 43. Bailly A, Sovero V, Vincenzetti V, Santelia D, Bartnik D, Koenig BW,
587 Mancuso S, Martinoia E, Geisler M (2008) Modulation of P-glycoproteins
588 by auxin transport inhibitors is mediated by interaction with immunophilins.
589 J Biol Chem **283**,21817-21826.
590 44. Geisler M, Kolukisaoglu HU, Bouchard R, Billion K, Berger J, Saal B, Frangne
591 N, Koncz-Kalman Z, Koncz C, Dudler R, et al. (2003) TWISTED DWARF1,
592 a unique plasma membrane-anchored immunophilin-like protein, interacts
593 with Arabidopsis multidrug resistance-like transporters AtPGP1 and
594 AtPGP19. Mol Biol Cell **14**,4238-4249.
595 45. Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular Aspects of
596 Seed Dormancy*. Annu. Rev. Plant Biol. **59**,387-415.

46. Schachtman DP, Goodger JQD (2008) Chemical root to shoot signaling under drought. Trends in Plant Science **13**,281-287.
47. Davidson AL, Dassa E, Orelle C, Chen J (2008) Structure, function, and evolution of bacterial ATP-binding cassette systems. Microbiol Mol Biol Rev **72**,317-364.
48. Lee M, Choi Y, Burla B, Kim Y-Y, Jeon B, Maeshima M, Yoo J-Y, Martinoia E, Lee Y (2008) The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO2. Nat Cell Biol **10**,1217-1223.
49. Yang H, Murphy AS (2009) Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in Schizosaccharomyces pombe. Plant J **59**,179-191.
50. Samuels L, Kunst L, Jetter R (2008) Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu Rev Plant Biol **59**,683-707.
51. Pighin JA, Zheng H, Balakshin LJ, Goodman IP, Western TL, Jetter R, Kunst L, Samuels AL (2004) Plant cuticular lipid export requires an ABC transporter. Science **306**,702-704.
52. Panikashvili D, Savaldi-Goldstein S, Mandel T, Yifhar T, Franke RB, Hofer R, Schreiber L, Chory J, Aharoni A (2007) The Arabidopsis DESPERADO/AtWBC11 transporter is required for cutin and wax secretion. Plant Physiol **145**,1345-1360.
53. Panikashvili D, Shi JX, Bocobza S, Franke RB, Schreiber L, Aharoni A (2009) The Arabidopsis DSO/ABCG11 Transporter Affects Cutin Metabolism in Reproductive Organs and Suberin in Roots. Mol Plant.
54. Luo B, Xue X-Y, Hu W-L, Wang L-J, Chen X-Y (2007) An ABC transporter gene of Arabidopsis thaliana, AtWBC11, is involved in cuticle development and prevention of organ fusion. Plant Cell Physiol **48**,1790-1802.
55. Bird D, Beisson F, Brigham A, Shin J, Greer S, Jetter R, Kunst L, Wu X, Yephremov A, Samuels L (2007) Characterization of Arabidopsis ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. Plant J **52**,485-498.
56. Osbourn AE (1996) Preformed Antimicrobial Compounds and Plant Defense against Fungal Attack. Plant Cell **8**,1821-1831.
57. Moons A (2008) Transcriptional profiling of the PDR gene family in rice roots in response to plant growth regulators, redox perturbations and weak organic acid stresses. Planta **229**,53-71.
58. Badri DV, Quintana N, El Kassis EG, Kim HK, Choi YH, Sugiyama A, Verpoorte R, Martinoia E, Manter DK, Vivanco JM (2009) An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiol **151**,2006-2017.
59. Badri DV, Loyola-Vargas VM, Broeckling CD, De-la-Pena C, Jasinski M, Santelia D, Martinoia E, Sumner LW, Banta LM, Stermitz F, et al. (2008) Altered profile of secondary metabolites in the root exudates of Arabidopsis ATP-binding cassette transporter mutants. Plant Physiol **146**,762-771.

60. Sugiyama A, Shitan N, Yazaki K (2008) Signaling from soybean roots to rhizobium: An ATP-binding cassette-type transporter mediates genistein secretion. Plant Signal Behav **3**,38-40.
61. Sugiyama A, Shitan N, Yazaki K (2007) Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume-Rhizobium symbiosis. Plant Physiol **144**,2000-2008.
62. Jasinski M, Stukkens Y, Degand H, Purnelle B, Marchand-Brynaert J, Boutry M (2001) A plant plasma membrane ATP binding cassette-type transporter is involved in antifungal terpenoid secretion. Plant Cell **13**,1095-1107.
63. Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A Putative ABC Transporter Confers Durable Resistance to Multiple Fungal Pathogens in Wheat. Science.
64. Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, et al. (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature **455**,195-200.
65. Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C, et al. (2008) Strigolactone inhibition of shoot branching. Nature **455**,189-194.

8. Figure legends

Figure 1. Arabidopsis ABC transporters in different organs with their substrate and/or function.

Figure 2. Tissue level localization of Arabidopsis ABC transporters with their substrates

(a) Mesophyll and epidermal cells. AtABCC1 and AtABCC2 reside on the tonoplast and mediate the transport of xenobiotic compounds and endogenous metabolites into the vacuole. AtABCG11/12 are polarly localized on the plasma membrane and export lipids used for cuticle formation. AtABCG36 is a resistance

674 factor for pathogens. **(b)** Guard cell. AtABCG40 acts as an ABA importer from the
675 apoplast into the cytosol and mediates ABA induced stomatal closure. The
676 tonoplast localized AtABCC5 transports $InsP_6$ (IP_6) into the vacuole thereby
677 lowering the active pool of $InsP_6$ in the cytosol. AtABCB14 is a plasma-
678 membrane malate importer that acts during stomatal movements. The major
679 outward current of malate during stomatal closure is indicated with a gapped blue
680 arrow. **(c)** Transection through a root. AtABCG25 is polarly localized on the
681 plasma membrane of parenchyma cells where it mediates ABA export from
682 parenchyma cells into the xylem stream. AtABCG36 has been shown to export
683 toxic cadmium ions that have unspecifically entered epidermal cells. AtABCG30
684 is an exporter for root exudates and resides on the plasma membrane of root
685 epidermal cells.
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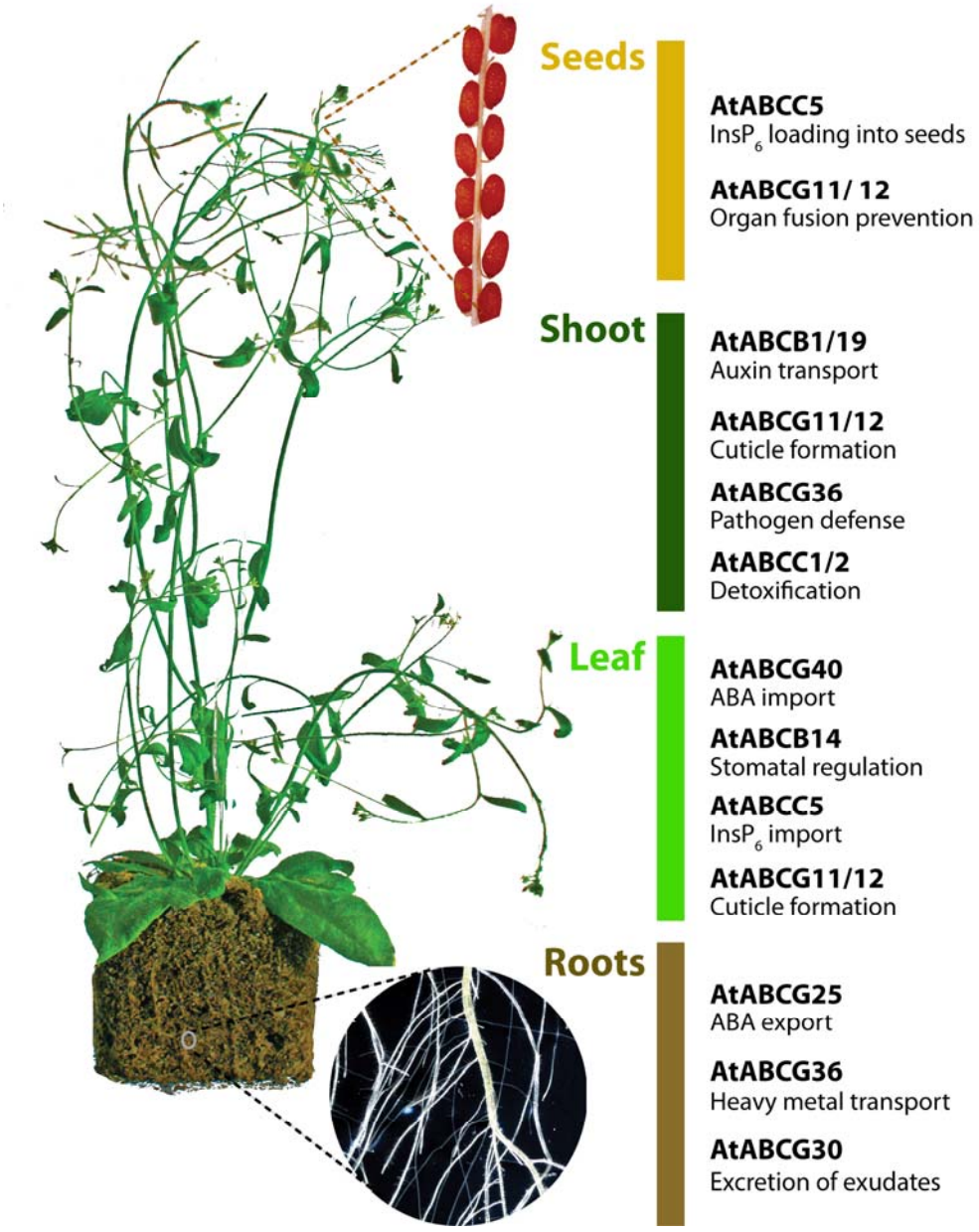
688 **9. Figure 1**



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10. Figure 2

10. Key words

Arabidopsis thaliana

ABCB/PGP subfamily

ABCC/MRP subfamily

Half-size ABCG/WBC subfamily

Full-size ABCG/PDR subfamily

Export and Import

Cellular detoxification

Phytate transport

Hormone transport

Stomatal movements,

Cuticle formation

Pathogen defense

Transport of natural compounds